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The bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply and utilize a unique shuttle, glycerophosphate oxidase, which includes a terminal oxidase, to reoxidize the glycolytically produced NADH. This terminal oxidase which is located in the mitochondrial membrane is cytochrome independent and not inhibited by classical inhibitors of the respiratory chain. This enzyme complex consists of two components: a flavin-linked glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphate oxidase (GPO) which are probably linked by ubiquinol. The oxidase component of the enzyme is absent in the mammalian host and is specifically inhibited by salicylhydroxamic acid. We describe here the partial purification of GPO from the bloodstream form of African trypanosomes. Mitochondria from Trypanosoma brucei bloodstream trypomastigotes were treated with 7.5 mM lauryl maltoside to release the oxidase from the mitochondrial membrane. Solubilized mitochondria was

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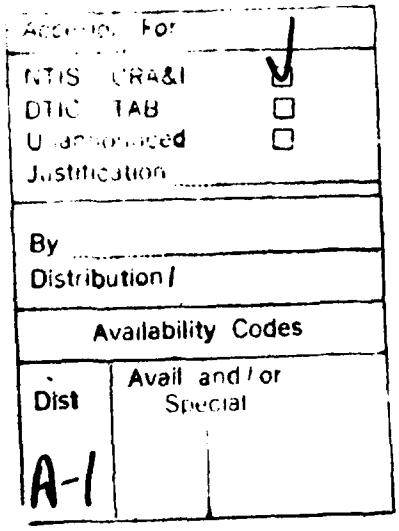
#19 ABSTRACT (Continued)

applied to a Pharmacia FPLC Mono Q HR 5/5 anion exchange column and chromatography was performed at 4. The oxidase was eluted from the column between 300 and 400 mM KCl, and a 22.4 fold enrichment for the oxidase was obtained. Enzyme activity was inhibited 100% by 0.5 mM SHAM, thus confirming the oxidase activity.



FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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ABSTRACT

The bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply and utilize a unique shuttle, glycerophosphate oxidase, which includes a terminal oxidase, to reoxidize the glycolytically produced NADH. This terminal oxidase which is located in the mitochondrial membrane is cytochrome independent and not inhibited by classical inhibitors of the respiratory chain. This enzyme complex consists of two components: a flavin-linked glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphate oxidase (GPO) which are probably linked by ubiquinol. The oxidase component of the enzyme is absent in the mammalian host and is specifically inhibited by salicylhydroxamic acid. We describe here the partial purification of GPO from the bloodstream form of African trypanosomes. Mitochondria from Trypanosoma brucei bloodstream trypomastigotes were treated with 7.5 mM lauryl maltoside to release the oxidase from the mitochondrial membrane. Solubilized mitochondria was applied to a Pharmacia FPLC Mono Q HR 5/5 anion exchange column and chromatography was performed at 4. The oxidase was eluted from the column between 300 and 400 mM KCl, and a 22.4 fold enrichment for the oxidase was obtained. Enzyme activity was inhibited 100% by 0.5 mM SHAM, thus confirming the oxidase activity.

Index Descriptions Abbreviations: Trypanosoma brucei; Glycerol-3-phosphate oxidase (GPO); Fast liquid protein chromatography (FPLC); Lauryl maltoside; SHAM, salicylhydroxamic acid

APPROACH TO THE PROBLEM

The electron transport system of African trypanosomes is a potential target for trypanocidal drugs. This is particularly true for bloodstream trypomastigotes that have a cyanide-insensitive α-glycerophosphate oxidase (GPO). This enzyme has not been purified or characterized in detail. Inhibition of this enzyme coupled with inhibition of the anaerobic glycolytic pathway would destroy the parasites.

The mammalian stage of the life cycle of African trypanosomes is primarily dependent on glycolysis for ATP production. No cytochromes are present. The brucei subgroup, which includes those forms that infect man and several animals, is exclusively dependent on glycolysis. They produce pyruvate from glucose under aerobic conditions and pyruvate and glycerol in equimolar amounts under anaerobic conditions.

The GPO is a cyanide - insensitive oxidase which is inhibited by hydroxamic acids including salicylhdroxamic acid (SHAM) (Clarkson and Brohn, 1976; Ryley, 1956, 1962, Grant et al. 1960). Unfortunately, administration of SHAM had little or no chemotherapeutic effect due to the presence of an anaerobic glycolysis scheme (Clarkson and Brohn, 1976; Evans and Brown, 1973; Evans et al. 1973; Opperdoes et al. 1976). A combination of SHAM and glycerol inhibits glycolysis in bloodstream trypomastigotes causing parasite destruction in vivo and in vitro.

Further identification of the biochemical properties of the GPO could be valuable as a chemotherapeutic approach.

BACKGROUND

Trypanosomes are flagellated protozoa responsible for several serious parasitic diseases of humans and domestic animals. Trypanosomes present various medical and economic obstacles for the development of several African and South American countries. African trypanosomiasis is ranked among the top six tropical diseases selected for scientific studies by the World Health Organization (Trigg 1979). These parasites are of interest to scientists not only because of their medical and veterinary importance but also because of several unique biochemical features. Bloodstream forms of Trypanosoma brucei and T. rhodesiense lack cytochromes (Flynn and Bowman 1973; Fairlamb 1982), are completely dependent on glycolysis for energy, and respiration in whole cells is insensitive to inhibitors such as cyanide, azide, and antimycin A (Flynn and Bowman 1973).

Early studies (von Brand and Tobie 1948) on the respiratory system of trypanosomes indicated the presence of a distinct type of respiratory enzyme system in the bloodstream form of Kinetoplastida. von Brand et al. (1950) also observed that the respiration of various bloodstream forms of trypanosomes was not inhibited but stimulated by KCN, thereby suggesting the presence of an alternative respiratory system.

Grant and Sargent (1961) were the first to observe a particulate L-α-glycerophosphate oxidase in the bloodstream form of T. rhodesiense, which would react with oxygen without the presence of either pyridine nucleotide enzymes or cytochromes. Fairlamb and Bowman (1977a) also suggested that during glycolysis the reoxidation of NADH was generated by means of an NAD dependent glycerol-3-phosphate dehydrogenase, which catalyzes the formation of glycerol-3-phosphate from dihydroxyacetone phosphate. Glycerol-3-phosphate is then reoxidized to the dihydroxyacetone phosphate by the mitochondrial glycerol-3-phosphate oxidase (GPO).

Like most cyanide insensitive electron transport systems which are found in certain plants and fungal mitochondria (Henry and Nys 1975), the GPO system can be completely inhibited by hydroxamic acids such as salicylhydroxamic acids (SHAM) (Opperdoes et al. 1976; Clarkson et al. 1981). Hill (1976) has shown that GPO has a lower affinity for oxygen than cytochrome oxidase (0.1 versus 2.1 uM) but it apparently reduces oxygen to water as the end product, since hydrogen peroxide could not be detected as a free intermediate (Caughey et al. 1979).

GPO, which is unique to the bloodstream forms of trypanosomes consist of two components: a flavin-linked glycerol-3-phosphate dehydrogenase (E.C.1.1.99.5) and an oxidase which are probably linked via ubiquinol (Fairlamb and Bowman 1977a). Studies using inhibitors of the GPO indicated that at least two enzyme components are present. Since the mitochondrial glycerol-3-phosphate dehydrogenase is capable of

transferring electrons either to the SHAM-sensitive terminal oxidase component or to an artificial electron acceptor (Fairlamb and Bowman 1977a, 1977b; Meshnick et al. 1978). Inhibitors acting after the site of reduction of the artificial electron acceptors were presumed to act on the terminal oxidase component (Fairlamb and Opperdoes 1986). The oxidase component deserves further investigation, as it is the enzyme which is absent in the mammalian host and is specifically inhibited by SHAM. This enzyme is therefore of interest as a possible target for drug chemotherapy. At present only suramin and organic arsenicals remain as the mainstay of chemotherapy, despite their many dangerous disadvantages.

With the use of a fast protein liquid chromatography (FPLC) system and a Mono Q anion exchange column, we have been able to solubilize and partially purify the GPO activity.

MATERIAL AND METHODS

Isolation of trypanosomes. Trypanosoma brucei EATRO 110 was obtained from Dr. M. R. Rifkin at Rockefeller University in New York. This strain was isolated in 1958 in East Africa from a naturally infected bovine and it has been cyclically passaged in tsetse flies and rodents. Male Sprague-Dawley (250-300 g) rats were inoculated intraperitoneally with $1x10^7$ cells /100 g weight. The animals were killed when the parasitemia level was between $8x10^8$ and $1x10^9$ cells/ml blood. The rats were anesthetized with ether and bled to death. A buffy coat was prepared and the trypanosomes were separated from the red blood cells on a DEAE cellulose column (Lanham 1968) eluted with phosphate buffered saline with glucose and heparin (PBSGH) at room temperature.

Preparation of parasite mitochondria. Parasites were pelleted by centrifugation in either a Sorvall HS-4 or GS-3 rotor for 5 min at 4,000 x g at 4^0C. The pellet was swollen in 1mM Tris and 1 mM EDTA pH 8.0, homogenized with a Teflon fitted hand homogenizer, and then passed three times through a 26 G 1/2" needle with 80 psi. Osmolarity was restored with the addition of sucrose, EDTA, and 0.5 M Tris, pH 7.5. Crude mitochondria was collected after centrifugation at 27,000 x g for 10 min. The pellet was then treated with 10 ug/ml DNAse I for 30 min at 4^0C. Mitochondria were isolated on a 20-35% renografin gradient as described by Braly et al. (1971).

Effect of lauryl maltoside on the release of GPO. Frozen

mitochondria were thawed and exposed to various concentrations (1-15 mM) of lauryl maltoside for 8 hr. The samples were centrifuged at 48,000 x g for 20 min at 4^0C and the supernatant fluids were assayed for enzyme activity. Control experiments consisted of exposing the mitochondria in buffer without detergent for the same amount of time followed by centrifugation at 48,000 x g for 20 min. Experiments with centrifugation at 100,000 x g yielded similar results.

Enzyme purification. Five mg of trypanosomal mitochondrial protein were exposed to 1 ml of loading buffer (7.5 mM lauryl maltoside, 10 mM K_2HPO_4, 5% sucrose, and 210 mM KCl, pH 7.5) for 2 hr at 4^0C. The Mono Q HR 5/5 anion exchange column (1.0 ml bed volume) was equilibrated with 5 ml running buffer A (7.5 mM lauryl maltoside, 10 mM K_2HPO_4, and 5% sucrose, pH 7.5), 5 ml runner buffer B (7.5 mM lauryl maltoside, 10 mM K_2HPO_4, 5% sucrose and 1M KCl, pH 7.5) then again with 5 ml running buffer A. Two mg of lauryl maltoside solubilized mitochondrial protein were loaded onto the anion exchange column and eluted with a 50 ml linear KCl gradient (0 to 1M) at a flow rate of 0.5 ml/min. The effluent was monitored at 280 nm in a FPLC UV absorbance detector set at 0.5 full scale deflection and 1 ml fractions were collected. All fractions were assayed for protein concentrations using the dye-binding method described by Bradford (1976).

Enzyme assay. Protein fractions eluted from the FPLC system were measured for enzyme activity with an Instech 125/05 oxygen electrode maintained at 25^0C by a circulating water bath. Oxygen consumption was

measured by a Yellow Spring Model 5300 oxygen monitor. The oxygen electrode chamber contained 0.5 ml of 40 mM Tris, pH 8.0 and 3 mg/ml bovine serum albumin. Oxidase activity was measured after the addition of 0.6 mM of the ubiquinol analog, 2,3,-dimethoxy-5-methyl-6-nonyl-1,4-benzoquinone which was synthesized according to Catlin et al. (1971) as the electron donor. To confirm the oxidase activity, 0.5 mM SHAM was used to inhibit the oxidase activity 100%.

RESULTS AND DISCUSSION

Effect of lauryl maltoside on GPO. Figures 1 and 2 show the amount of GPO released from the mitochondria membrane after treatment with lauryl maltoside. The highest amount of activity released from the mitochondria membrane occurred after exposure to a concentration of 7.5 mM lauryl maltoside for four hours. After 2 hours of exposure to the detergent, specific activity for the oxidase was observed to be 24 nmoles O_2 consumed/min/mg protein. Exposure to the detergent lauryl maltoside after 4 hours increased the specific activity to 28 nmoles O_2 consumed/min/mg protein. However, it was also observed that after 4-8 hours of exposure (fig.3), enzyme activity for the oxidase decreased 75%. No enzyme activity was detected in the supernatant fluids of the controls indicating that the activity measured was released from the mitochondria after detergent treatment.

Enzyme purification. A summary of the partial purification of the ubiquinol oxidase is shown in Table 1. This procedure resulted in an overall recovery of 13.4% and a 22.4 fold purification. Eighty percent of the protein was recovered from the column with 60% of the protein eluted in the void volume. The oxidase was eluted from the column between 300 and 400 mM KCl. Fractions containing the enzyme were totally inhibited by the addition of SHAM to the reaction mixture.

Enzyme assays. The elution profile of the oxidase is shown in figure 4. All fractions containing proteins were assayed for oxidase activity.

The first five fraction that contained 60% (0.8-1.2 mg) of the loaded protein were negative for the oxidase. Proteins eluted from the anion exchange column between 300 and 400 mM KCl (fractions 19, 20, and 21) contained 30.0, 30.0 and 10.0 ug of protein, respectively (fig.4). No enzyme activity was detected in fraction 19. The specific activity of fraction 20 was 560 nmoles O_2 consumed/min/mg protein and that of fraction 21 was 420 nmoles O_2 consumed/min/mg protein. Enzyme activity in both fractions were inhibited 100% by the addition of 0.5 mM SHAM.

Partial purification of GPO can be obtained with the use of a FPLC system and a Mono Q anion exchange column. After the addition of 2 mg of solubilized mitochondrial proteins with specific activity of 16 nmoles O_2 consumed/min/mg protein were applied to the anion exchange column, the oxidase was eluted between 300 and 400 mM KCl. Fractions containing 30 ug of protein had a specific activity of 560 nmoles O_2 consumed/min/mg protein for the oxidase resulting in a 22.4 fold enrichment for the oxidase. These results also indicate that frozen mitochondria can be used for these experiments with success. Apparently prolonged freezing is not detrimental to the oxidase. However, if the oxidase is stored overnight at 4^0C, enzyme activity can not be detected. This indicates that the oxidase is unstable once removed from the mitochondrial membrane.

The major problem encountered with this purification scheme is the initial loss of activity after centrifugation. Approximately 60% of the enzyme is pelleted with mitochondrial membranes, clearly revealing

that the detergent does not lyse the mitochondria completely. This occurs as a result of selecting a mild but effective detergent which is effective in releasing hydrophobic proteins. One advantage of using this purification scheme is that a small amount of material is required. Therefore the use of trypanosomal mitochondria is practical, for with 1 mg of solubilized mitochondrial protein the FPLC monitor can detect the protein peak containing the oxidase activity.

This is the first successful report on the partial purification of the oxidase from the mitochondrial membrane of T. brucei. Tielens and Hill (1985) reported that with the aid of affinity chromatography they were able to bind a fraction of T. brucei protein to the affinity column and that the oxidase was eluted from the column with borate but the oxidase activity could not be detected. Fairlamb and Bowman (1977a) were able to partially purify GPO 24 fold from lysed T. brucei with a combination of differential and isopycnic sucrose gradient centrifugation, but since no detergent was used the enzyme was probably still bound to the membrane.

Enzyme assays for GPO have shown that the oxidase is a part of GPO. Enzyme activity of the oxidase was detected in the holoenzyme with the addition of a ubiquinol analog or with the substrate glycerol-3-phosphate. However, when the holoenzyme is solubilized the oxidase activity can only be measured with the addition of the ubiquinol analog. This suggests that the oxidation of glycerol-3-phosphate and

ubiquinol analogs are not two independent processes but are reactions of the same enzyme complex. The purification of the oxidase from the mitochondrial membrane of T. brucei supports the studies of Opperdoes and Borst (1977) who suggested that the cyanide insensitive respiratory chain is localized in the mitochondria and is probably the main enzyme system responsible for reoxidizing the NADH generated in aerobic glycolysis. The NADH is reoxidized by a NAD-dependent glycerol-3-phosphate dehydrogenase and then the reducing equivalents are oxidized by the cyanide-insensitive glycerol-3-phosphate oxidase located in the mitochondria as described by Fairlamb and Bowman (1977b). The electron carrier between the two enzymes is unknown. However, it appears to be a ubiquinone-like mediator which aids in the transfer of electrons between the two enzymes. This is supported by the use of ubiquinol analogues which restore the oxidase activity after the GPO complex has been solubilized with detergents. Similar observations were reported by Tielens and Hill (1985), who used ubiquinol analogs to measure the oxidase activity of GPO from mitochondria treated with octylglucoside.

Lauryl maltoside is similar to octylglucoside except for the presence of an alkyl side chain. This detergent has been used to solubilize cytochrome oxidase from mitochondria of other organisms (Dutch et al. 1987). It was observed that optimum release of the enzyme occurred at a concentration of 7.5 mM detergent after four hours of exposure. Tielens and Hill (1985) observed that the optimal concentration for octylglucoside was 2% and 1% for deoxycholate which resulted in a 30-

60% recovery of enzyme activity. However, it is interesting that prolonged exposure to a 1mM concentration of lauryl maltoside caused a decrease in enzyme activity. Another disadvantage of the detergent lauryl maltoside is that it does not completely separate the holoenzyme for there is some activity of the GPO complex after 4 hours at a concentration of 15 mM. Tielens and Hill (1985) reported that after samples were treated with octylglucoside no activity for the holoenzyme could be detected.

The results of the enzyme purification indicates that after the mitochondria had been solubilized with 7.5 mM lauryl maltoside, a 30 fold enrichment of the oxidase could be obtained. Several investigators (Fairlamb and Bowman 1977a; Tielens and Hill 1985) have isolated either the oxidase or the dehydrogenase from trypanosomes using conventional protein purification methods. When enzymes assays were carried out on lysed whole cells and treated with various concentration of lauryl maltoside the oxidase was inhibited 98-100%. Concentrations as low as 1 mM lauryl maltoside inhibited enzyme activity approximately 98%, suggesting the release of proteins which probably either block or degrade the oxidase activity. The results presented indicate that this procedure can serve as a preliminary step for the purification of the oxidase. Purification of the enzyme will provide a protein complex available for inhibition studies. Current studies are devoted to the identification of protein bands associated with the enzyme activity.

REFERENCES

Bradford M. M. 1976. Anal. Biochem. 72, 248-254.

Braly, P., Simpson, L. and Kretzer, F. 1971. J. Protozool. 21, 782.

Catlin, J.C., Daves C. D., and Folkers, K. 1971. J. Med. Chem. 14, 45-48.

Caughey, B., Hill, G. C. and Rich, Pl 1979. J. Protozool. 26, 8A.

Clarkson, A. and Brohn, F., 1976. Science 194:204-206.

Clarkson, A. B., Jr. Grady, R.W., Grossman, S.A., MacCallhum, R.J. and Brohn, F. H. 1981. Mol. Biochem. Parasitol. 3, 217-291.

Dutch, R., Ingle, R., Centeno, J., Peiffer, W., Babcock, G. and Ferguson-Miller, S. 1987. Plant Mitochondria (Moore, A. L. and Beechery, R. B., ed) pp. 301-304, Plenum Publishing Corporation, New York City.

Evans, D. A. and Brown, R. C. 1973. J. Protozool. 20:157-160.

Evans, D. A., Brightman, C. J. and Holland 1973. M. F. Lancet 2, 769.

Fairlamb, A. H. 1982. Trends. Biochem. Sci. 7, 249-253.

Fairlamb, A. H. and Bowman, I. B. R. 1977a. Int. J. Biochem. 8, 669-675.

Fairlamb, A. H. and Bowman, I. B. R. 1977b. Int. J. Biochem. 8, 659-668.

Fairlamb, A. H. and Opperdoes, F. P. 1986. Carbohydrate Metabolism in Cultured Cells (M. J. Morgan ed.) pp. 183-244. Plenum Publishing Corporation, New York City.

Flynn, I. W. and Bowman, I. B. R. 1973. Comp. Biochem. Physiol. 45B, 25-42.

Grant, P. T. and Sargent, Jr. R. 1960. Biochem. J. 76:229-237.

Grant, P. T. and Sargent 1961. Biochem. J. 81, 204-214.

Henry, M. F. and Nyns, E. J. 1975. Sub-Cell Biochem. 4, 1-65.

Hill, G. C. 1976. Biochem. Biophys. Acta 456, 149-193.

Lanham, S. M. 1968. Nature (Lond). 218, 1273-1274.

Meshnick, S. R., Blobstein, S. H., Grady, R. W. and Cerami, A. 1978. J. Exp. Med. 148, 569-579.

Opperdoes, F. R. and Borst, P. 1977. FEBS Letters 80, 360-364.

Opperdoes, F. R., Borst, P. and Fonck, K. 1976. FEBS Letters 62, 169-172.

Opperdoes, F. R., Aarson, P. N., van der Meer, C., and Borst, P. 1976. Exp. Parasit. 40:198-205.

Ryley, J. F. 1956. Biochem. J. 62:215-222.

Ryley, J. F. 1962. Biochem. J. 85:211-223.

Tielens, A. G. M. and Hill, G. C. 1985. J. Parasit. 71, 384-386.

Trigg, P. I. 1979. Trends Biochem. Sci. 4, 29-30.

von Brand, T. and Tobie, E. J. 1948. J. Cell Comp. Physiol. 31, 49-68.

von Brand, T., Tobie, E. J. and Mehlman, B. 1950. J. Cell. Comp. Physiol. 35, 273-300.

Explanation of figures

Figure 1. The effect of various concentrations of lauryl maltoside on the release of the enzyme GPO from the mitochondria of T. brucei after 2 hrs of detergent treatment. Enzyme assays were on the supernatant fluid after a 48,000 x g centrifugation for 20 min. No enzyme activity was observed in the controls, (□—□) GPO, (◇—◇) UBQO, (⊡—⊡) G-3PDH.

Figure 2. The effect of various concentrations of lauryl maltoside on the release of T. brucei GPO after 4 hrs of exposure to the detergent, (□—□) GPO, (◇—◇) UBQO, (⊡—⊡) G-3PDH.

Figure 3. A time course of T. brucei GPO after exposure to 7.5 mM lauryl maltoside for 8 hours, (□—□) GPO, (◇—◇) UBQO, (⊡—⊡) G-3PDH .

Figure 4. Elution profile of proteins and GPO from solubilized T. brucei mitochondria after FPLC anion exchange chromatography. The enzyme was eluted as described in the text. The arrow indicates the addition of 2 mg solubilized mitochondria protein on to the Mono Q HR 5/5 anion exchange column (△—△) protein, (□—□) GPO.

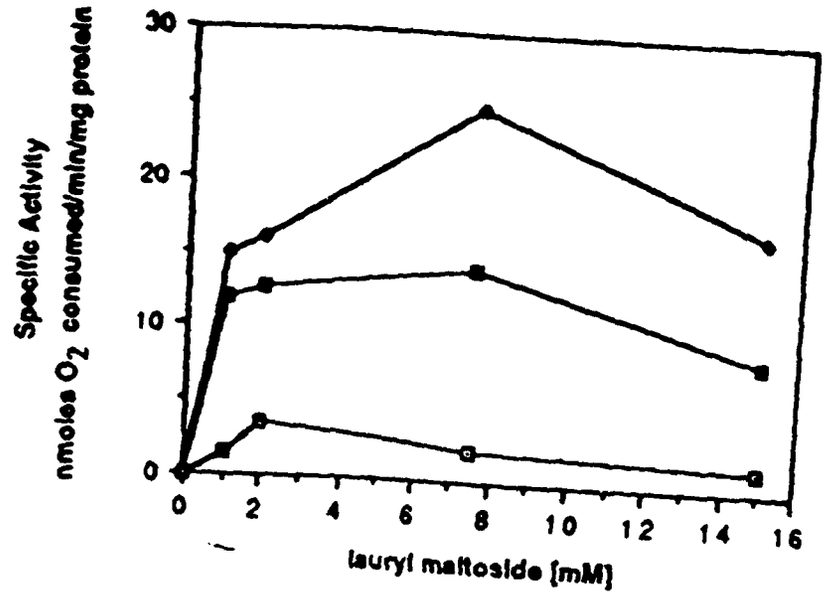

Specific Activity
nmoles O_2 consumed/min/mg protein
30
20
10
0
0
2
4
6
8
10
12
14
16
lauryl maltoside [mM]

Figure 1

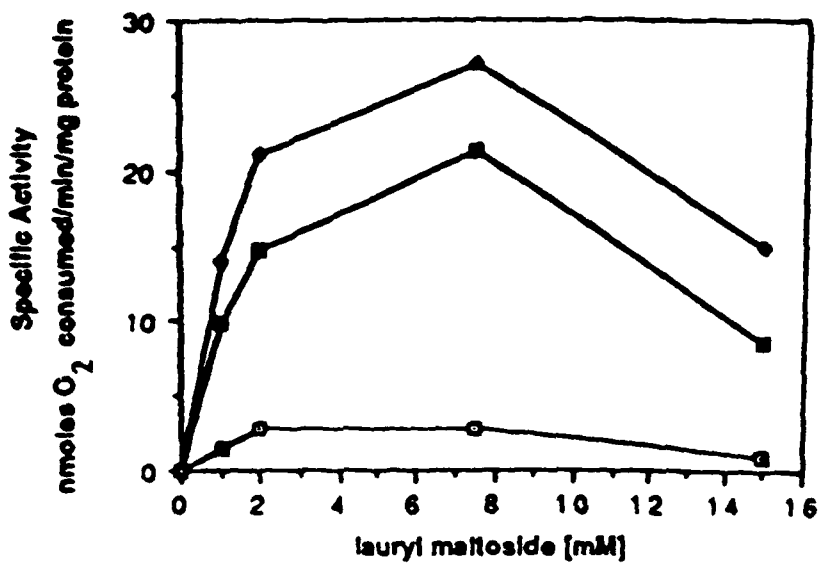
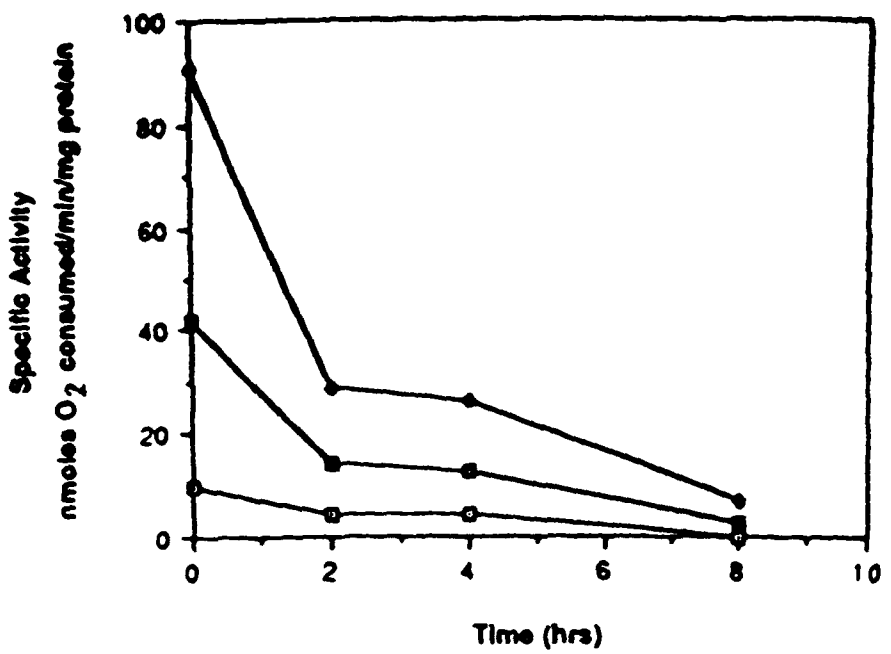

Specific Activity
nmoles O_2 consumed/min/mg protein
30
20
10
0
0
2
4
6
8
10
12
14
16
lauryl maltoside [mM]

Figure 2


Specific Activity
nmoles O_2 consumed/min/mg protein
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80
60
40
20
0
0
2
4
6
8
10
Time (hrs)

Figure 3

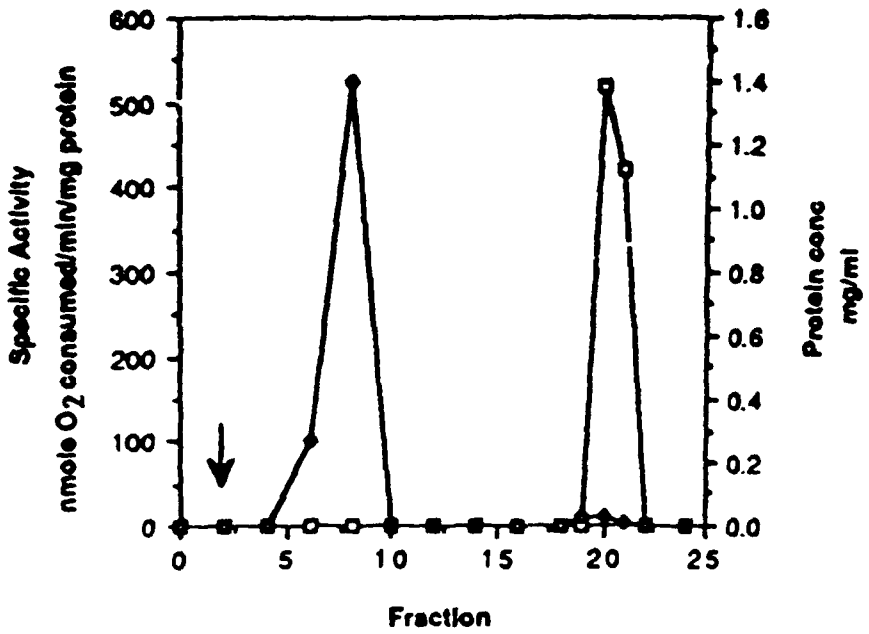
Specific Activity
nmole O_2 consumed/min/mg protein
Protein conc
mg/ml
Fraction
600
500
400
300
200
100
0
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25

Figure 4

TABLE 1

Partial Purification of Ubiquinol Oxidase from *Trypanosoma brucei*

Step	Total protein (mg)	Specific activity nmoles O_2 /min/ mg protein	Enzyme activity nmoles O_2 / min	Purification	Percent Yield
Mitochondria	5.0	25.0	125.0	1.0	100.0
Solubilized Mitochondria Protein*	2.0	16.0	32.0	0.64	25.0
Mono Q**	0.03	560.0	16.8	22.4	13.4

* Protein released as a result of 7.5 mM lauryl maltoside.

** Pharmacia anion exchange column.